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Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women

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Abstract

Most epidemiology studies investigating potential adverse health effects in relation to phthalates measure the urinary concentration of the free plus glucuronidated species of phthalate metabolites (i.e., total concentration) to estimate exposure. However, the free species may represent the biologically relevant dose. In this study, we collected 943 urine samples from 112 men and 157 women and assessed the between- and within-person variability and predictors of a) the free and total urinary concentrations of phthalate metabolites, and b) the percentage of free phthalate metabolites (a potential phenotypic indicator of individual susceptibility). We also explored the proportion of urinary di-(2-ethylhexyl) phthalate (DEHP) metabolites contributed to by the bioactive mono-2-ethylhexyl phthalate (MEHP), considered a possible indicator of susceptibility to phthalate exposure. The percentage of phthalate metabolites present in the free form were less stable over time than the total metabolite concentration, and, therefore, it is not likely a useful indicator of metabolic susceptibility. Thus, the added costs and effort involved in the measurement of free in addition to total metabolite concentrations in large-scale studies may not be justified. Conversely, the proportion of DEHP metabolites contributed to by MEHP was more stable within individuals over time and may be a promising indicator of susceptibility if time of day of sample collection is carefully considered.

Keywords

biomarker; environment; exposure; metabolism

INTRODUCTION

Human exposure to phthalates is widespread (CDC, 2011), and a growing number of recent studies have suggested that exposure to phthalates at concentrations found among the general population may be associated with a range of adverse health effects (Hauser and Calafat, 2005; Meeker et al, 2009; Swan, 2008). The most common method for assessing exposure to phthalates is measurement of phthalate metabolites in urine because it offers

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many advantages over measuring the diesters or their metabolites in blood. The advantages include: ease of sample collection, larger sample volumes of urine vs blood, higher concentrations of the metabolites, and reduced potential for contamination by the parent diester and subsequent formation of metabolites by enzymes present in blood (Koch and Calafat, 2009). Following exposure to the parent phthalate diesters, diesters are rapidly cleaved into their corresponding hydrolytic monoesters by esterases and lipases (Frederiksen et al, 2007; Lyche et al, 2009; Silva et al, 2003). The monoester metabolites then: 1) undergo phase II biotransformation to form glucuronide-conjugated monoesters that are excreted in the urine; 2) go through phase I biotransformation reactions (e.g., oxidation) to form more hydrophilic secondary oxidized metabolites prior to glucuronidation; and/or 3) a portion of the unconjugated (free) monoester and/or secondary metabolites may also be directly excreted in urine.

As far as we are aware, all epidemiologic studies to date that have utilized urinary biomarkers measured the concentration of the free plus glucuronidated species of phthalate metabolites (i.e., total concentration). However, because the metabolites are more bioactive than the parent diester, and the free form of the metabolite may be more bioactive than the glucuronidated form, it has been hypothesized that measurement of the free metabolite concentration may be a better metric of biologically effective dose (Silva et al, 2003). Furthermore, the ratio of free to total phthalate metabolites in urine may vary among individuals (Silva et al, 2003). If these ratios were stable over time within individuals, it could indicate that some people may be more efficient in glucuronidation and excretion of phthalate metabolites (i.e., phase II metabolism) and thus potentially less susceptible to effects related to phthalate exposure.

The present study was designed to assess the between- and within-person variability and predictors (related to demographics and the timing of urine sample collection) of free and total urinary phthalate metabolite concentrations, as well as the percentage of free phthalate metabolite present in the urine. A high degree of temporal reliability in the percentage of the metabolite present in its free form within individuals over time may suggest that it is useful as a phenotypic marker of phase II metabolism efficiency.

In addition to glucuronidation, another factor related to metabolism that may play a role in the toxicity following exposure to phthalates (especially high molecular weight phthalates such as di-(2-ethylhexyl) phthalate [DEHP]) prior to phase II biotransformation is the efficiency with which phthalate monoester metabolites are further metabolized to produce more hydrophilic oxidized metabolites (Barr et al, 2003; Silva et al, 2003) . Because mono-2-ethylhexyl phthalate (MEHP) is bioactive, we hypothesized in our earlier work that the ratio of urinary MEHP to the secondary DEHP metabolites (mono-2-ethyl-5-hydroxyhexyl phthalate [MEHHP], mono-2-ethyl-5-oxohexyl phthalate [MEOHP], mono-2-ethyl-5-carboxypentyl phthalate [MECPP]), referred to as MEHP%, could represent a person's relative efficiency to form the more hydrophilic and potentially less biologically active secondary metabolites (Ferguson et al, 2011; Hauser et al, 2007; Hauser, 2008; Meeker et al, 2007; Meeker et al, 2009). We hypothesized that MEHP% may be a marker of an individual's susceptibility to DEHP exposure. In the present study, we investigated predictors of MEHP% and its temporal reliability within a person over time, which, if high, would support the use of this ratio as a marker of phase I metabolic susceptibility to DEHP exposure.

Finally, we also assessed temporal variability and predictors of the ratio between two secondary metabolites of DEHP (MECPP and MEHHP), which has recently been proposed as a marker of the timing of exposure to DEHP (Lorber et al. 2010). The MECPP:MEHHP ratio may reflect timing of exposure due to the longer estimated elimination half-life of

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MECPP (12-15 hours) compared to MEHHP (~ 10 hours) (Koch et al, 2006), where a lower MECPP:MEHHP ratio would represent a more recent exposure to DEHP.

METHODS

Study population

Study participants were male and female partners seeking infertility evaluation and treatment at the Massachusetts General Hospital Fertility Center. They were recruited between November 2004 and February 2008. Partners underwent ovulation induction with timed intercourse or timed intrauterine insemination and assisted reproductive technologies, which included in vitro fertilization and intracytoplasmic sperm injection. Fertility Center couples that conceived naturally were also enrolled. Subjects were followed from recruitment throughout their treatment cycles until either a live birth or the discontinuation of treatment.

Men and women between the ages of 18 to 55 and 18 to 45 years, respectively, were eligible. Men who had undergone a vasectomy were ineligible. Most study patients cited the lack of time as the primary reason for not participating. A research nurse administered a questionnaire to collect data on date of birth, race/ethnicity, medical history, smoking history, and lifestyle factors. The study was approved by the Human Studies Institutional Review Boards of the Massachusetts General Hospital, Harvard School of Public Health, the Centers for Disease Control and Prevention, and the University of Michigan. Subjects signed an informed consent after the study procedures were explained and all questions answered.

Urine sample collection

Both men and women provided a spot urine sample at the time of recruitment and at subsequent visits during treatment cycles, as well as at post-treatment clinical appointments. If applicable, women also collected three spot urine samples during pregnancy, one during each trimester. Urine was collected in a non-sterile clean polypropylene container. Specific gravity (SG) was measured using a handheld refractometer (National Instrument Company Inc., Baltimore, MD). The urine was then divided in aliquots and frozen at −80°C. Samples were shipped overnight to the CDC on dry ice for measurement of phthalate metabolites.

Urinary phthalate metabolites

The analytical methods used to quantify urinary phthalate metabolite concentrations have been described elsewhere (Blount et al, 2000; Kato et al, 2005; Silva et al, 2003; Silva et al, 2004; Silva et al, 2007). Briefly, they involved enzymatic deconjugation of the metabolites from their glucuronidated form (this step was omitted when measuring the concentrations of free species), solid-phase extraction, separation with high performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry. Detection limits varied slightly depending on the analytical method used (Blount et al, 2000; Kato et al, 2005; Silva et al, 2003; Silva et al, 2004; Silva et al, 2007) for each phthalate metabolite (monoethyl phthalate [MEP], 1.00 to 1.21 ng/ml; MEHP, 0.87 to 1.20 ng/ml; MEHHP, 0.7 to 1.60 ng/ml; MEOHP, 0.6 to 1.20 ng/ml; MECPP, 0.25 to 0.5 ng/ml; mono-3-carboxypropyl phthalate [MCPP], 0.16 to 0.37 ng/ml; mono-carboxyoctyl phthalate [MCOP], 0.7 ng/ml and mono-carboxynonyl phthalate [MCNP], 0.5 ng/ml). Isotopically-labeled internal standards and conjugated internal standards were used to increase precision of measurements. Along with the study samples, each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentration to monitor for accuracy and precision. Analysts at the CDC were blind to all information concerning subjects. MCOP and MCNP were added to the analytical method mid-study and

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thus were measured in a smaller number of samples than the other metabolites. Metabolite concentrations below the limit of detection (LOD) were assigned a value of LOD divided by 2. For analyses utilizing SG-corrected metabolite concentrations the following formula was used:

$$P_c = P[(1.024 - 1)/(SG - 1)] \quad [1]$$

where P_c is the SG-adjusted phthalate metabolite concentration (nanograms per milliliter), P is the observed phthalate metabolite concentration, and SG is the specific gravity of the urine sample.

Free MEHP was not measured because most MEHP is excreted in urine as a conjugate (Silva et al, 2003). The small proportion of free MEHP in urine (geometric mean = 16 %) and the relatively low urinary concentrations of MEHP species (free plus conjugated ~2 ng/ml) result in a large number of non-detectable concentrations of free MEHP (Silva et al, 2003).

Several “biotransformation metrics” were calculated using the free and total metabolite concentrations for the statistical analysis. First, the percentage of free metabolite to total metabolite concentration (%free) was calculated for all metabolites for which we had data on both free and total forms (MEHHP, MEOHP, MECPP, MEP, MCPP, MNCP and MCOP). Second, for DEHP, we created a variable to represent the percentage of the sum of concentrations of DEHP metabolites that was measured as MEHP (MEHP%). This was done by converting the concentrations of the four DEHP metabolites MEHP, MEHHP, MEOHP, and MECPP into nanomoles (nmol), dividing the molar mass of MEHP by the mass of the sum of all four metabolites, and then multiplying by 100. Finally, the mass concentration ratio of MECPP to MEHHP was calculated as a hypothesized indicator of DEHP exposure timing (Lorber et al, 2010).

Statistical analysis

Data analysis was performed using SAS version 9.2 for Windows (SAS Institute Inc., Cary, NC). Spearman rank correlations were calculated to assess relationships between all metabolites and biotransformation metrics. Descriptive statistics and distributions were tabulated for free and total metabolite concentrations, and for the calculated biotransformation indicators, and compared between categories for sex, age, pre-pregnancy BMI, race, smoking, pregnancy status, season, and time of day at urine sample collection. Because the data involved repeated measures, differences in natural log (ln)-transformed metabolite concentrations between categories were tested statistically using linear mixed effects models with a random subject effect to account for potential correlation of measurements within an individual. These models were fit for each metabolite (or biotransformation indicator) and covariate pairing individually; full models for each metabolite/indicator when including all covariates simultaneously were also constructed. To assess between- and within-person variability (i.e. temporal reliability) in urinary metabolite concentrations and biotransformation indicators, intraclass correlation coefficients (ICC) and their 95% confidence intervals were calculated (Hankinson et al, 1995). In a secondary analysis ICCs were also calculated when including only samples collected within 30 days of one another, and when excluding samples collected from women during pregnancy.

Finally, to explore whether phthalate metabolite concentrations and biotransformation indicators were correlated between male and female partners within a couple, we explored scatterplots and Spearman correlations among the subset of urine samples that were collected from the male and female partner on the same day and within the same time category.

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RESULTS

A total of 269 participants (112 men and 157 women) were included in the study. Each participant contributed between 1 and 15 urine samples (mean 3.4 samples, median 3 samples), and the time lapsed between repeated samples within a person ranged from several days to several months (median 35 days). Participants were mostly white (87%), non-smokers (96%), and their ages ranged from 21 to 45 years (mean 35.4, median 35.0) among women and from 26 to 54 years (mean 35.0, median 34.0) among men. Of the 688 urine samples collected from 157 women in the study, 123 samples from 75 of the women were collected during pregnancy. For the assessment of within-couple correlations there were 140 paired male and female urine sample concentrations from the same couple on the same day within the same time category.

The distribution of urinary free and total phthalate metabolites were presented in Table 1, and the distribution of biotransformation metrics (%free metabolite concentrations, MEHP %, and MECPP:MEHHP ratio) were presented in Table 2. Among the metabolites, MEP was present at the highest concentrations for both free (median 69.5 ng/ml) and total (median 96.2 ng/ml) concentrations, followed by MECPP (median = 19.6 ng/ml free, 47.5 ng/ml total). MEP was also the metabolite found to have the highest proportion of its total urinary excretion in the free form (median = 77%). The four carboxylates (MCPP, MCNP, MCOP, and MECPP) had median %free values between 40 and 60%, whereas the median %free for MEHHP and MEOHP was considerably lower (6%). Values for %free varied considerably across and within individuals for all metabolites, as did MEHP% and MECPP:MEHHP, suggesting heterogeneity in these metrics of biotransformation within this population.

Using Spearman correlation analyses (data not shown), for a given phthalate metabolite there were strong correlations between urinary free and total metabolite concentrations, with correlation coefficients ranging from 0.77 for MEOHP to 0.96 for MECPP. For a given phthalate metabolite, only weak correlations were observed between free metabolite concentration and %free (all Spearman $r < 0.12$). When comparing total metabolite concentrations to %free, weak to moderate inverse correlations were found for a given metabolite (Spearman r ranged from -0.01 for MEP to -0.52 for MEOHP).

Concentrations of SG and free and total metabolites, stratified by demographic and sampling characteristics, were presented in Table 3. Because SG was significantly associated with sex, BMI, smoking status, and time of day of sample collection, the SG-corrected phthalate metabolite concentrations were shown in Table 3. Free and total metabolite concentrations differed significantly by time of day of sample collection for all metabolites except MCNP and MCOP. For the secondary DEHP metabolites (MEHHP, MEOHP, MECPP) and MCPP, urine samples collected later in the day (between 3 and 6PM) had the highest concentrations, followed by samples collected before 9AM. On the other hand, urine samples collected between 3 and 6PM had the lowest concentrations of both free and total MEP. Women had higher concentrations of free MEOHP (but not of the other secondary DEHP metabolites) and MCPP compared to men, but total concentrations of these metabolites did not differ by sex. Among women in the study, samples collected during pregnancy had higher total MEHP and total MCPP, but lower total and free MEP, compared to samples not collected during pregnancy. Both free and total MEP concentrations differed by age group, where there was evidence for an increasing trend in MEP concentrations with age for participants older than 30 years of age. BMI categories were associated with greater total (but not free) MEHHP and MECPP concentrations. Finally, non-white participants had higher free and total MEP concentrations and higher total MEHP concentrations than white participants.

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Predictors of the phthalate biotransformation indicators (%free, MEHP%, MECPP:MEHHP ratio) were presented in Table 4. Women had higher %free for MEHP, MEOHP, and MEP compared to men. Conversely, men had higher %free for MCPP and MCOP. Among women, samples collected during pregnancy had higher MEHP% and %free MEHHP and % free MEOHP, but lower %free MECPP and MECPP:MEHHP ratio, compared to samples not collected during pregnancy. BMI categories were inversely associated with %free for MEHHP, MEOHP, and MEP, and with MEHP%. Time of day that the urine sample was collected was associated with most of these indicators. There was an increasing trend for %free MECPP with samples collected later in the day, and a concomitant decreasing trend with MECPP:MEHHP ratio. There was also a significant increasing trend in MEHP% with time of day. For several of the other metabolites (MEHHP, MEOHP, and MEP), the percentage of free metabolite concentrations was greatest for samples collected between 9AM and 12PM. In full linear mixed effects models adjusted for all covariates (sex, age, BMI, race, smoking, season and time of day of sample collection), all of the associations described for free and total concentrations of the metabolites, and biotransformation indicators remained statistically significant (data not shown), with the exception of the associations of BMI with total MECPP concentrations and the percentage of free MEP. Among women, significant associations between pregnancy status and total MEP, free MEP, total MCPP, and %free MECPP remained in full models adjusted for all covariates, whereas the relationships with total MEHP and %free for MEHHP and MEOHP were no longer statistically significant.

The ICCs for free and total metabolite concentrations, and biotransformation indicators, were presented in Table 5. Since ICCs were similar when using uncorrected or SG-corrected free and total phthalate metabolites concentrations, we chose to present ICCs uncorrected for SG in the table. The ICC for SG was 0.26 (95%CI 0.19, 0.34). The ICCs for the free and total concentrations of all metabolites, with the exception of MEP, were weak (≤0.24) and suggest poor temporal reliability in these measures. The ICCs for both free and total concentrations of MEP were considerably stronger (0.50). However, the ICC for %free was weak for MEP (0.22) and all the other metabolites (ranging between 0.04 and 0.26). Finally, the ICC for MEHP% was 0.39 (95%CI 0.32, 0.47), suggesting moderate yet higher temporal reliability for this biotransformation indicator compared to the other indicators.

In secondary analyses we calculated ICCs when excluding samples collected from women during pregnancy, and also calculated ICCs when excluding samples collected more than 30 days apart. Results with these exclusions were similar to those among the full dataset (not shown). The largest change in ICC when excluding pregnant women was observed for MEHP%, which increased from 0.39 to 0.44. The largest change in ICC when restricting to samples collected within 30 days of one another was for %free MEP, which increased from 0.22 to 0.34.

Among the paired urine samples within couples from male and female partners collected on the same day (n=140), there were moderate positive correlations between male and female for all SG-corrected free and total metabolite concentrations (not shown; Spearman r ranged from 0.27 for free MEP to 0.42 for total MEHP). There were no correlations between male and female partners for %free for all metabolites (all r > 0 but <0.15), with the exception of a weak correlation for %free MCPP (r=0.23). Likewise, MEHP% (r = -0.02) and MECPP:MEHHP ratio (r = -0.01) were not correlated within couple.

DISCUSSION

In the present study, the concentrations of total (i.e., free plus glucuronidated) urinary species of phthalate metabolites in men and women were similar to those reported among

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the U.S. general population (CDC, 2011). However, concentrations of MEP were lower and concentrations of DEHP metabolites were higher in this study compared to NHANES. For example, the median (95th percentile) metabolite concentrations were (in ng/ml) 96.2 (1750), 4.8 (107), 32.3 (502), and 47.5 (658) for MEP, MEHP, MEHHP, and MECPP, respectively, compared to 128 (2230), 2.1 (27.3), 20.4 (214), and 29.2 (286), respectively, among participants aged 20 years and older in NHANES 2007-2008. These differences in phthalate metabolite concentrations between the two populations may be due to differences related to demographics, lifestyle choices (e.g., diet, personal care products usage) and/or timing of urine sample collection.

To our knowledge, only one study has measured free urinary phthalate metabolites in a human population previously (Silva et al, 2003). Similar to the present study, the median %free MEP was 79% in that study compared with 77% in our data. The high %free for MEP was not unexpected because, compared to more nonpolar and lipophilic phthalate metabolites (e.g. MEHP), more hydrophilic metabolites such as MEP are more likely to be rapidly excreted in urine before undergoing phase II metabolism (Silva et al, 2003). Free and total MEHP, monobutyl phthalate and monobenzyl phthalate were the other metabolites measured by Silva and colleagues. However, due to low concentrations and detection frequency of these metabolites in their free forms, they were not included in the present study. Our study adds to the Silva et al work by measuring free and total concentrations of additional phthalate metabolites, specifically the secondary metabolites of DEHP, as well as MCNP, MCOP and MCPP, and by assessing the temporal reproducibility of concentrations in repeated urine samples collected from the same individuals over time.

Nearly all free and total concentrations of metabolites measured in the present study, as well as %free, were associated with time of day of urine collection. For the total concentrations of metabolites, this is consistent with previous reports (Preau et al, 2010; Silva et al, 2004). The primary route of exposure to DEHP for most adults is considered to be the diet (Wormuth et al, 2006). Thus, our finding of greater free and total urinary concentrations of DEHP metabolites between 3 – 6PM may reflect exposure resulting from meals eaten earlier in the day. For DEP, the primary source of exposure may be personal care products (Duty et al, 2005; Just et al, 2010; Preau et al, 2010). Our observation of the greatest free and total concentrations of MEP, the main DEP metabolite, in the middle of the day, and the lowest concentrations in late afternoon/evening, may reflect more personal care/hygiene product use in the morning compared to other times during the day. The elimination half-life of MEP is assumed to be 2-3 hours (Calafat and McKee, 2006). With this short half-life and because a large proportion of MEP does not undergo phase II biotransformation, the greatest amount of MEP may then be excreted rapidly in the hours following exposure which may be during the middle of the day for most people. We also found that free and total MEP concentrations were lower in urine samples collected from women during pregnancy, which may reflect differences in DEP-containing product use while pregnant among this population of women. Finally, free and total MEP concentrations were higher among non-whites compared to whites, which is consistent with total MEP concentrations reported in NHANES 1999-2008 (CDC, 2011; Silva et al, 2004).

The ICCs for total concentrations reported here, which were weak for all metabolites besides the modest temporal reliability demonstrated by MEP (which may reflect consistent personal hygiene product use patterns within a person), were somewhat consistent with those presented in earlier research (Fromme et al, 2007; Hauser et al, 2004; Hoppin et al, 2002; Peck et al, 2010; Preau et al, 2010; Teitelbaum et al, 2008). However, ICCs reported by previous studies have varied, possibly due to differences in study population, time of day urine samples were collected, and interval between sample collections For all metabolites measured in the present study, including MEP, we also found weak ICC values for %free.

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This suggests the percentage of the metabolite present in the free form is not stable over time within individuals. Thus, this does not support our hypothesis that %free may serve as a phenotypic marker of individual susceptibility to phthalate exposure due to less efficient phase II metabolism. On the other hand, a stronger ICC (lower within-person variability) was found for MEHP%, a hypothesized marker of phase I metabolism (Hauser, 2008). In support of this hypothesis and the patterns we observed was a study that reported a higher ICC for MEHP% (ICC=0.60) compared to MEHP (ICC = 0.35) in repeated urine samples collected from pregnant women (Adibi et al, 2008). However, given the observed trend in MEHP% by time of day of sample collection in an inverse pattern to that of the relationship between time of day and MECPP:MEHHP ratio, as well as the differences in biologic half-lives between MEHP and the secondary metabolites, it is likely that timing since last exposure and timing of the previous urination are also important contributors to MEHP%, apart from differences in metabolism both within and between persons.

We observed moderate within-couple correlations for free and total phthalate metabolite concentrations in urine samples collected from male and female partners on the same date/time. This may represent some similarities in diet, personal care product use, indoor environment, and other potential exposure sources within couples. Conversely, we found that MEHP% and MECPP:MEHHP ratio were not correlated within couple. For MEHP% this may further support our hypothesis that it represents a potential indicator of individual metabolic susceptibility rather than purely timing of DEHP exposure. However, the MECPP:MEHHP ratio, which is thought to be an indicator of DEHP exposure timing, was also not correlated within couple. This may be due to differences in dietary patterns (i.e., foods eaten and timing of meals) between partners within a couple, or could be related to differences in metabolism by sex.

Strengths of our study included its large size and the collection of repeated urine samples from the same participants over time. Limitations of our study included the collection of spot urine samples (as opposed to collecting 24-hour void samples), unknown time of last urination, and limited data to allow for the determination of sources, pathways and activities related to phthalate exposure. However, a recent study reported that the collection of first-morning and 24-hour urine samples did not reduce the within-individual variability in MEHHP over the course of one week compared to spot urine samples (Preau et al, 2010). Another potential limitation of our study relates to the inability to generalize our results to other populations, especially children and infants who may not have fully mature glucuronidation pathways (Lyche et al, 2009) and may be otherwise more susceptible to health effects related to exposure.

In conclusion, as reported previously by us and others, there is high temporal variability in phthalate metabolite concentrations within individuals over time. For this reason, investigators are encouraged to collect detailed temporal information related to lifestyle activities (e.g., diet, personal hygiene practices) and multiple urine samples from each study participant for the assessment of phthalate exposure in a manner consistent with the goals and outcome measures of interest in a particular study. In addition, most studies rely on spot urine samples due to logistical issues surrounding more time-integrated samples. Since time of day that urine samples are collected is important, investigators are also encouraged to collect urine samples within carefully selected time windows during the day, if possible, and record information on time of sample collection and time of the participant's previous urine void. Finally, while the urinary concentration of free species of phthalate metabolites may be a marker of a person's biologically relevant dose, %free is less stable over time than the total concentration of metabolites and %free is not likely useful as a phenotypic indicator of metabolic susceptibility. Thus, unless free metabolite concentrations are demonstrated to be a superior estimate of biologically effective dose in future studies, measurement of free in

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addition to total metabolite concentrations in large-scale studies may not be worth the added effort and expense involved. On the other hand, MEHP% may still be a promising indicator of phase I biotransformation if sample collection is uniformly timed in epidemiologic studies and deserves further consideration.

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Table 1

Distribution of free and total phthalate metabolite concentrations (ng/ml)

Phthalate Metabolite	N	Geometric Mean	Selected Percentiles						
			10th	25th	50th	75th	90th	95th	Max
Free MEHHP	943	2.5	<LOD	0.9	2.5	6.4	18.6	32.5	358
Free MEOHP	943	1.7	<LOD	<LOD	1.6	4.0	11.2	19.3	148
Free MECPP	943	21.0	3.1	7.9	19.6	56.6	149	305	5,580
Free MEP	943	81.6	11.2	25.3	69.5	249	727	1,290	27,200
Free MCPP	943	1.1	0.1	0.5	1.3	2.7	6.2	15.3	225
Free MCNP	519	1.3	<LOD	<LOD	1.2	2.7	7.1	19.3	355
Free MCOP	519	3.2	<LOD	1.3	3.2	8.1	21.2	40.7	709
Total MEHHP	943	32.4	4.1	10.6	32.3	93.0	274	502	8,780
Total MEOHP	943	21.0	2.7	6.9	21.0	62.1	174	314	3,410
Total MECPP	943	50.2	6.9	17.6	47.5	142	366	658	7,170
Total MEP	943	111	16.3	36.3	96.2	329	937	1,750	32,900
Total MCPP	943	2.1	0.3	0.8	2.2	4.9	10.9	25.3	607
Total MCNP	519	2.5	<LOD	1.1	2.5	4.9	11.7	38.2	1,680
Total MCOP	519	7.0	1.2	2.8	6.4	16.3	51.9	94.7	2,250
Total MEHP	943	5.3	<LOD	1.7	4.8	14.3	49.5	107	1,710

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Table 2

Distribution of phthalate biotransformation metrics: percent free phthalate metabolite concentrations, percentage of measured DEHP metabolites present as MEHP (MEHP%), and the ratio of MECPP to MEHHP.

Phthalate Metabolite	N	Geometric Mean	Selected Percentiles						
			10th	25th	50th	75th	90th	95th	Max
Free MEHHP%	943	8	3	4	6	11	44	65	100
Free MEOHP%	943	8	3	4	6	12	44	76	100
Free MECPP%	943	42	25	31	41	56	74	83	100
Free MEP%	943	74	55	68	77	88	96	98.6	100
Free MCPP%	943	57	33	47	59	75	100	100	100
Free MCNP%	519	52	23	36	58	88	100	100	100
Free MCOP%	519	47	28	36	47	62	92	100	100
MEHP%	943	5	2	3	5	8	11	14	29
MECPP:MEHHP ratio	943	1.6	1.0	1.2	1.5	2.0	2.7	3.3	12.8

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Table 3

Median (25th, 75th) specific gravity (SG) and SG-corrected free and total phthalate metabolite concentrations by select variables (ng/ml)

	# subjects	# samples	SG	Free MEHHP	Free MEOHP	Free MECPP	Free MEP	Free MCPP	Free MCNP[c]	Free MCOP[c]
Overall	269	943	1.016 (1.009, 1.022)	4.1 (1.7, 11.0)	2.6 (1.4, 6.2)	33.1 (15.2, 78.0)	124 (49.3, 412)	2.6 (1.4, 6.2)	2.1 (1.2, 4.6)	5.3 (2.4, 12.0)
Sex										
Female	157	688	1.015 (1.007, 1.021)	4.2 (1.7, 11.3)	2.8 (1.5, 7.0)	33.2 (14.9, 76.5)	134 (55.2, 418)	2.8 (1.5, 7.0)	2.1 (1.2, 5.1)	5.1 (2.1, 12.3)
Male	112	255	1.020 (1.012, 1.024)	3.5 (1.5, 9.7)	1.9 (0.9, 5.0)	32.9 (16.1, 81.3)	112 (40.9, 391)	1.9 (0.9, 5.0)	2.1 (1.2, 3.5)	5.7 (2.9, 11.7)
p-value[a]			<0.0001	0.15	0.0003	0.11	0.37	0.0003	0.80	0.52
Pregnant[b]										
No	157	565	1.016 (1.007, 1.022)	4.3 (1.8, 11.4)	2.8 (1.5, 7.2)	33.6 (15.0, 74.0)	140 (57.5, 436)	2.8 (1.5, 7.2)	2.1 (1.2, 5.3)	4.9 (2.1, 11.8)
Yes	75	123	1.011 (1.007, 1.020)	3.8 (1.7, 10.0)	3.1 (1.4, 5.7)	31.4 (14.7, 107)	104 (38.4, 287)	3.1 (1.4, 5.7)	2.2 (1.1, 4.6)	5.7 (2.4, 18.0)
p-value[a]			0.29	0.31	0.72	0.49	0.0007	0.72	0.46	0.29
Age										
<30	21	110	1.016 (1.010, 1.024)	4.2 (1.7, 8.9)	2.7 (1.4, 5.6)	31.3 (13.8, 76.4)	166 (60.0, 1097)	2.7 (1.4, 5.6)	2.2 (1.0, 3.6)	5.8 (3.4, 13.2)
30-34	85	308	1.015 (1.007, 1.021)	3.8 (1.7, 12.2)	2.5 (1.3, 8.2)	32.5 (15.7, 81.6)	96.2 (37.3, 325)	2.5 (1.3, 8.2)	2.2 (1.2, 4.5)	4.8 (2.1, 10.4)
35-39	108	335	1.016 (1.009, 1.022)	4.1 (1.7, 11.3)	2.7 (1.4, 6.4)	36.0 (14.7, 72.6)	113 (46.8, 428)	2.7 (1.4, 6.4)	2.1 (1.2, 5.2)	5.2 (2.3, 12.0)
40-44	43	138	1.020 (1.009, 1.024)	3.5 (1.8, 8.3)	2.2 (1.3, 5.1)	31.3 (15.9, 84.3)	185 (74.8, 426)	2.2 (1.3, 5.1)	1.8 (0.9, 6.0)	5.2 (2.6, 12.3)
>=45	10	42	1.019 (1.015, 1.024)	3.9 (2.2, 10.8)	2.5 (1.4, 5.0)	33.2 (15.2, 56.4)	174 (97.8, 534)	2.5 (1.4, 5.0)	5.2 (2.6, 12.3)	11.6 (4.9, 18.5)
p-value[a]			0.55	0.97	0.87	0.99	0.001	0.87	0.96	0.57
BMI										
<24.9	134	511	1.015 (1.007, 1.021)	4.0 (1.7, 11.0)	2.6 (1.4, 6.7)	31.5 (13.9, 71.6)	122 (55.0, 373)	2.6 (1.4, 6.7)	2.1 (1.2, 4.8)	5.3 (2.0, 12.3)
25 – 29.9	83	252	1.018 (1.010, 1.023)	4.1 (1.7, 9.9)	2.5 (1.2, 5.2)	32.9 (16.7, 84.8)	112 (41.8, 380)	2.5 (1.2, 5.2)	2.3 (1.3, 3.7)	4.9 (2.8, 10.8)
>=30	48	155	1.020 (1.012, 1.025)	4.2 (1.8, 11.7)	2.7 (1.3, 7.3)	37.4 (16.4, 96.0)	197 (43.8, 686)	2.7 (1.3, 7.3)	1.9 (1.1, 4.8)	7.0 (2.6, 13.2)
p-value[a]			0.002	0.85	0.32	0.29	0.25	0.32	0.92	0.33
Race										
Non-white	34	100	1.020 (1.012, 1.024)	5.2 (1.9, 9.1)	3.0 (1.7, 6.4)	33.7 (17.1, 89.3)	182 (55.8, 618)	3.0 (1.7, 6.4)	1.9 (1.2, 3.4)	5.4 (3.6, 18.2)
White	233	833	1.016 (1.008, 1.022)	4.0 (1.7, 11.2)	2.5 (1.4, 6.2)	32.7 (15.0, 77.5)	122 (48.5, 391)	2.5 (1.4, 6.2)	2.2 (1.2, 4.7)	5.3 (2.3, 11.6)

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	# subjects	# samples	SG	Free MEHHP	Free MEOHP	Free MECPP	Free MEP	Free MCPP	Free MCNP[c]	Free MCOP[c]
p-value[a]			0.07	0.66	0.55	0.29	0.02	0.55	0.30	0.18
Smoking										
Non- Smoker	257	903	1.016 (1.008, 1.022)	4.1 (1.7, 11.3)	2.6 (1.4, 6.4)	32.9 (15.0, 79.7)	127 (50.0, 409)	2.6 (1.4, 6.4)	2.1 (1.2, 4.5)	5.2 (2.4, 12.3)
Smoker	12	40	1.021 (1.017, 1.026)	3.5 (1.7, 7.2)	2.2 (1.1, 5.0)	36.6 (19.7, 65.7)	74.6 (31.4, 799)	2.2 (1.1, 5.0)	2.5 (1.7, 7.2)	7.4 (3.4, 9.7)
p-value[a]			0.005	0.19	0.20	0.86	0.80	0.20	0.24	0.82
Season										
Winter	131	213	1.018 (1.009, 1.023)	3.8 (1.7, 8.6)	2.4 (1.3, 5.0)	30.8 (15.0, 69.1)	118 (46.9, 398)	2.4 (1.3, 5.0)	2.1 (1.0, 3.8)	5.3 (2.1, 10.2)
Spring	121	206	1.015 (1.008, 1.023)	3.6 (1.7, 9.9)	2.8 (1.3, 5.9)	31.7 (14.9, 67.2)	147 (56.0, 432)	2.8 (1.3, 5.9)	2.4 (1.2, 5.7)	6.4 (3.0, 17.0)
Summer	145	263	1.016 (1.008, 1.022)	4.2 (1.8, 13.0)	2.8 (1.5, 7.7)	37.8 (17.5, 89.3)	111 (45.3, 426)	2.8 (1.5, 7.7)	2.2 (1.3, 4.8)	4.9 (2.2, 11.7)
Fall	147	261	1.016 (1.009, 1.022)	4.2 (1.7, 11.7)	2.5 (1.3, 7.1)	31.2 (14.3, 88.1)	151 (45.2, 390)	2.5 (1.3, 7.1)	1.9 (1.1, 3.5)	5.2 (2.4, 12.0)
p-value[a]			0.51	0.42	0.34	0.37	0.39	0.34	0.40	0.47
Time of Day										
6– 8:59AM	145	300	1.020 (1.011, 1.024)	4.2 (1.8, 10.8)	2.7 (1.4, 6.8)	36.9 (16.4, 82.0)	118 (49.0, 385)	2.7 (1.4, 6.8)	2.0 (1.0, 4.3)	5.3 (2.4, 11.6)
9 – 11:59AM	185	392	1.015 (1.008, 1.022)	3.8 (1.7, 11.0)	2.4 (1.4, 5.7)	30.4 (13.8, 63.8)	150 (58.4, 460)	2.4 (1.4, 5.7)	2.0 (1.2, 4.5)	5.3 (2.4, 12.3)
12 – 2:59PM	131	152	1.013 (1.006, 1.021)	2.6 (1.4, 8.2)	1.8 (1.1, 4.2)	29.4 (14.7, 62.4)	163 (45.2, 394)	1.8 (1.1, 4.2)	2.5 (1.2, 5.2)	5.2 (1.7, 14.2)
3 – 6PM	79	98	1.015 (1.008, 1.021)	6.5 (2.6, 20.3)	4.0 (1.7, 11.4)	50.4 (21.4, 207)	82.1 (38.4, 279)	4.0 (1.7, 11.4)	2.3 (1.0, 4.7)	5.3 (2.7, 10.8)
p-value[a]			<0.0001	0.002	0.002	<0.0001	0.006	0.002	0.65	0.86
	# subjects	# samples	Total MEHP	Total MEHHP	Total MEOHP	Total MECPP	Total MEP	Total MCPP	Total MCNPc	Total MCOPc
Overall	269	929	8.0 (3.2, 23.2)	51.7 (23.7, 134)	33.9 (14.5, 88.3)	82.9 (36.3, 197)	173 (71.0, 512)	3.4 (1.9, 6.7)	3.8 (2.3, 7.5)	10.8 (5.5, 24.8)
Sex										
Female	157	688	8.2 (3.3, 24.4)	52.5 (23.1, 131)	36.2 (14.5, 88.9)	82.7 (35.8, 188)	178 (74.1, 510)	3.6 (1.9, 6.8)	4.2 (2.3, 8.0)	10.6 (5.1, 28.0)
Male	112	241	7.3 (3.1, 20.1)	50.6 (24.3, 144)	30.9 (14.5, 85.2)	83.1 (37.0, 211)	151 (60.3, 522)	3.3 (1.9, 6.2)	4.2 (1.9, 6.9)	12.8 (5.9, 32.5)
p-value[a]			0.98	0.21	0.93	0.09	0.67	0.86	0.44	0.85
Pregnant[b]										
No	157	565	8.0 (3.2, 22.2)	52.4 (23.9, 127)	35.5 (14.4, 86.4)	84.0 (37.5, 194)	182 (78.2, 564)	3.4 (1.9, 6.7)	4.1 (2.2, 8.2)	10.4 (5.0, 27.8)
Yes	75	123	10.0 (3.6, 34.4)	52.8 (22.2, 144)	37.2 (16.5, 108)	77.9 (30.7, 182)	139 (63.5, 381)	3.8 (2.4, 7.6)	4.6 (2.9, 7.4)	13.2 (5.6, 29.8)

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	# subjects	# samples	SG	Free MEHHP	Free MEOHP	Free MECPP	Free MEP	Free MCPP	Free MCNP[c]	Free MCOP[c]
p-value[a]			0.02	0.55	0.18	0.61	0.0007	0.003	0.86	0.21
Age										
<30	21	110	8.0 (2.9, 32.1)	53.2 (24.0, 142)	32.2 (12.8, 96.2)	87.8 (35.2, 192)	228 (79.3, 1530)	4.2 (1.9, 6.9)	3.6 (2.1, 6.2)	12.8 (5.9, 32.5)
30-34	85	306	7.2 (3.1, 22.0)	49.5 (21.0, 138)	33.1 (13.4, 89.2)	80.3 (35.1, 184)	125 (52.4, 423)	3.3 (1.9, 6.8)	3.8 (2.4, 7.5)	10.2 (4.6, 22.7)
35-39	108	333	8.1 (3.4, 23.9)	50.9 (21.3, 125)	36.0 (14.4, 83.7)	84.9 (37.0, 197)	153 (64.0, 557)	3.4 (1.9, 6.2)	4.2 (2.3, 8.2)	10.9 (6.0, 23.8)
40-44	43	138	8.7 (3.6, 23.0)	54.0 (25.9, 137)	35.2 (1 7.2, 86.4)	74.5 (40.5, 236)	223 (104, 606)	3.2 (2.0, 6.3)	4.2 (2.4, 10.0)	11.6 (6.0, 30.4)
>=45	10	42	9.1 (3.4, 17.0)	57.7 (34.2, 143)	39.5 (19.0, 77.0)	86.1 (38.5, 209)	257 (155, 630)	4.1 (2.4, 8.5)	3.8 (2.3, 6.8)	22.3 (7.8, 51.4)
p-value[a]			0.88	0.95	0.99	0.98	0.0005	0.84	0.91	0.56
BMI										
<24.9	134	509	8.2 (3.2, 23.0)	47.3 (20.4, 116)	31.5 (13.8, 79.9)	76.6 (32.8, 178)	174 (71.8, 463)	3.4 (1.9, 6.7)	3.8 (2.2, 8.2)	10.4 (4.8, 25.4)
25 – 29.9	83	251	7.1 (3.0, 25.9)	56.1 (25.9, 146)	36.5 (16.1, 101)	91.2 (42.8, 209)	152 (67.8, 474)	3.4 (2.0, 6.1)	3.8 (2.5, 6.7)	10.5 (5.7, 21.3)
>=30	48	154	8.9 (3.5, 21.4)	57.1 (27.6, 175)	37.6 (13.4, 101)	87.0 (38.2, 236)	269 (71.8, 1001)	3.6 (2.2, 7.1)	4.0 (2.2, 6.8)	14.0 (7.0, 27.0)
p-value[a]			0.77	0.03	0.22	0.05	0.10	0.22	0.88	0.21
Race										
Non-white	34	99	11.1 (5.1, 32.7)	53.8 (25.9, 160)	33.2 (16.3, 109)	80.7 (40.6, 251)	284 (74.0, 774)	3.6 (2.1, 6.5)	3.8 (2.2, 6.0)	11.7 (7.7, 32.0)
White	233	830	7.7 (3.0, 22.9)	50.9 (22.7, 132)	33.9 (14.4, 87.0)	83.0 (35.4, 192)	170 (69.6, 471)	3.4 (1.9, 6.7)	3.8 (2.4, 7.6)	10.8 (5.1, 24.5)
p-value[a]			0.03	0.34	0.31	0.30	0.03	0.49	0.36	0.17
Smoking										
Non- Smoker	257	890	7.8 (3.1, 23.6)	51.8 (23.5, 138)	33.9 (14.4, 88.8)	83.5 (35.8, 197)	176 (71.8, 508)	3.4 (1.9, 6.7)	3.8 (2.3, 7.4)	10.8 (5.3, 25.4)
Smoker	12	39	11.5 (4.5, 23.0)	49.1 (24.5, 109)	33.2 (16.3, 83.7)	68.4 (43.9, 168)	104 (42.1, 1180)	4.2 (2.4, 6.9)	6.8 (2.8, 8.4)	12.3 (6.6, 19.3)
p-value[a]			0.96	0.46	0.57	0.64	0.81	0.29	0.40	0.83
Season										
Winter	131	211	8.9 (3.1, 20.4)	52.8 (22.4, 121)	35.2 (13.2, 79.3)	76.6 (35.2, 186)	166 (69.4, 508)	3.6 (1.9, 7.0)	3.8 (2.0, 7.8)	10.8 (4.5, 22.6)
Spring	121	201	7.4 (3.1, 21.4)	46.8 (21.0, 120)	32.1 (15.0, 84.0)	76.8 (35.3, 174)	195 (80.4, 572)	3.2 (1.8, 7.4)	4.3 (2.6, 8.6)	13.2 (6.3, 29.8)
Summer	145	258	8.1 (3.6, 29.5)	58.0 (26.2, 149)	41.9 (16.9, 99.6)	93.1 (40.2, 221)	167 (62.2, 498)	3.7 (2.2, 6.8)	4.3 (2.7, 7.6)	9.8 (5.4, 30.2)
Fall	147	259	7.2 (2.9, 23.6)	47.2 (24.4, 132)	29.3 (13.6, 87.6)	84.9 (33.8, 197)	177 (67.5, 471)	3.3 (1.9, 6.1)	3.4 (2.3, 6.6)	10.6 (5.6, 24.8)
p-value[a]			0.41	0.50	0.24	0.38	0.46	0.39	0.21	0.65

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	# subjects	# samples	SG	Free MEHHP	Free MEOHP	Free MECPP	Free MEP	Free MCPP	Free MCNP[c]	Free MCOP[c]
Time of Day										
6– 8:59AM	145	299	8.7 (3.6, 23.0)	61.8 (27.7, 152)	43.2 (18.3, 101)	93.1 (42.7, 253)	172 (70.9, 488)	3.8 (2.2, 7.3)	4.1 (2.4, 7.5)	11.7 (6.0, 25.7)
9 – 11:59AM	185	382	7.2 (3.0, 15.7)	45.1 (19.5, 106)	27.9 (12.9, 69.2)	74.0 (30.9, 168)	184 (80.2, 600)	3.4 (1.8, 6.4)	3.7 (2.1, 7.4)	10.7 (4.8, 26.0)
12 – 2:59PM	131	150	6.2 (2.9, 20.5)	43.9 (20.9, 102)	27.6 (12.6, 68.0)	62.7 (32.0, 152)	201 (63.6, 512)	2.6 (1.8, 5.2)	4.1 (2.6, 7.2)	9.5 (5.2, 22.5)
3 – 6PM	79	97	18.4 (6.0, 92.3)	110 (36.4, 363)	63.1 (24.4, 233)	138 (51.0, 387)	102 (50.4, 313)	3.9 (2.4, 9.1)	3.8 (2.2, 7.7)	12.0 (6.8, 23.3)
p-value[a]			<0.0001	<0.0001	<0.0001	<0.0001	0.004	0.009	0.87	0.88

[a]Test for fixed effects using log-transformed data in mixed models with random subject effects;

[b]Female samples only.

[c]Measured in 519 samples from 176 people

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Table 4

Median (25th, 75th percentile) biotransformation indicators stratified by select variables: free to total phthalate metabolite percentages, percentage of measured DEHP metabolites present as MEHP (MEHP%), and ratio of MECPP to MEHHP.

	# subjects	# samples	Free MEHHP%	Free MEOHP%	Free MECPP%	Free MEP%	Free MCPP%	Free MCNP%[c]	Free MCOP%[c]
Overall	269	943	5.8 (4.2, 10.9)	5.9 (3.8, 12.1)	41.4 (31.4, 55.9)	77.2 (67.5, 88.1)	58.8 (46.9, 75.0)	58.3 (36.4, 87.5)	47.1 (35.6, 61.5)
Sex									
Female	157	688	6.1 (4.3, 13.4)	6.2 (4.0, 15.6)	41.4 (31.3, 56.6)	78.5 (69.1, 89.1)	57.1 (44.8, 75.0)	54.9 (35.0, 90.9)	44.0 (34.6, 60.9)
Male	112	255	5.2 (4.0, 8.4)	5.1 (3.5, 8.5)	41.6 (31.7, 54.5)	74.7 (61.3, 84.3)	61.4 (51.6, 75.0)	63.9 (39.1, 82.6)	51.9 (40.4, 61.5)
p-value[a]			0.002	0.0003	0.89	0.0005	0.002	0.36	0.02
Pregnant[b]									
No	157	565	6.1 (4.3, 14.4)	6.2 (4.1, 17.4)	40.4 (29.6, 54.7)	78.6 (68.7, 89.1)	57.1 (44.7, 73.5)	55.3 (35.3, 92.6)	43.8 (34.4, 62.3)
Yes	75	123	5.8 (4.0, 10.3)	5.9 (3.7, 11.3)	46.7 (36.9, 63.0)	77.6 (69.5, 89.1)	60.0 (45.2, 75.0)	54.6 (31.9, 87.7)	44.7 (36.0, 55.0)
p-value[a]			0.02	0.02	0.001	0.88	0.82	0.22	0.69
Age									
<30	21	110	5.7 (4.3, 9.6)	5.5 (3.6, 13.5)	40.3 (30.0, 56.8)	77.1 (67.7, 87.7)	58.5 (46.9, 74.1)	62.0 (35.0, 93.3)	47.0 (38.3, 65.9)
30-34	85	308	6.4 (4.1, 14.2)	6.1 (3.9, 15.7)	42.1 (31.9, 61.3)	78.4 (67.1, 89.5)	60.0 (47.4, 75.5)	58.3 (37.0, 91.8)	48.2 (35.6, 64.4)
35-39	108	335	5.9 (4.4, 10.9)	5.9 (4.2, 12.1)	42.1 (32.0, 55.2)	77.5 (68.5, 87.0)	58.3 (46.3, 73.0)	55.6 (37.5, 82.1)	45.8 (35.4, 59.4)
40-44	43	138	5.2 (3.9, 9.2)	5.8 (3.6, 10.2)	40.8 (29.0, 55.7)	77.0 (68.7, 88.1)	58.0 (45.2, 82.1)	57.8 (27.3, 83.3)	48.5 (33.6, 63.2)
>=45	10	42	5.6 (4.1, 7.5)	5.1 (3.5, 8.7)	38.1 (32.8, 44.6)	73.6 (65.2, 83.3)	58.2 (52.6, 65.3)	48.3 (33.3, 64.7)	47.8 (36.0, 60.5)
p-value[a]			0.66	0.69	0.79	0.95	0.89	0.60	0.74
BMI									
<24.9	134	511	6.4 (4.4, 15.2)	6.3 (4.2, 17.1)	42.5 (33.2, 59.0)	79.0 (68.7, 90.3)	59.4 (45.5, 75.6)	59.4 (37.2, 89.2)	46.3 (35.4, 62.2)
25 – 29.9	83	252	5.4 (4.0, 7.9)	5.3 (3.6, 8.8)	41.1 (30.0, 53.5)	76.8 (66.7, 84.6)	58.6 (49.8, 73.9)	60.2 (34.6, 82.7)	50.0 (38.4, 60.1)
>=30	48	155	4.9 (3.8, 9.2)	5.5 (3.4, 11.5)	40.4 (30.0, 53.3)	75.1 (62.3, 84.5)	58.7 (47.4, 71.4)	52.7 (36.4, 87.5)	46.5 (33.3, 60.8)
p-value[a]			0.001	0.002	0.13	0.03	0.95	0.56	0.64
Race									
Non-white	34	100	5.5 (4.5, 8.4)	5.8 (3.8, 9.7)	41.4 (29.3, 55.3)	77.7 (69.2, 87.9)	60.0 (48.2, 79.1)	60.5 (37.5, 82.1)	47.5 (35.0, 61.7)
White	233	833	5.9 (4.1, 11.7)	5.9 (3.8, 13.5)	41.6 (31.5, 56.0)	77.4 (67.3, 88.1)	58.6 (46.8, 75.0)	58.1 (36.1, 87.5)	46.9 (35.6, 61.5)

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	# subjects	# samples	Free MEHHP%	Free MEOHP%	Free MECPP%	Free MEP%	Free MCPP%	Free MCNP%[c]	Free MCOP%[c]
p-value[a]			0.44	0.49	0.83	0.22	0.76	0.70	0.99
Smoking									
Non- Smoker	257	903	5.9 (4.1, 11.4)	5.9 (3.8, 12.5)	41.3 (30.8, 56.1)	77.6 (67.6, 88.1)	58.6 (46.7, 75.0)	58.3 (36.0, 87.5)	46.7 (35.4, 61.5)
Smoker	12	40	5.2 (4.3, 9.0)	5.6 (4.3, 8.5)	42.3 (37.5, 52.1)	72.5 (65.2, 82.7)	64.7 (50.0, 81.3)	62.5 (47.1, 82.7)	52.1 (46.5, 61.5)
p-value[a]			0.52	0.42	0.41	0.65	0.51	0.32	0.23
Season									
Winter	131	213	5.9 (4.0, 9.3)	5.9 (3.7, 12.3)	39.4 (30.4, 53.5)	76.7 (66.0, 86.2)	60.5 (50.0, 77.8)	60.7 (36.1, 90.5)	47.3 (37.0, 60.8)
Spring	121	206	5.8 (4.1, 12.2)	5.9 (3.6, 12.1)	40.2 (31.4, 54.6)	76.0 (67.7, 88.8)	59.3 (46.2, 80.0)	64.3 (36.0, 92.4)	51.3 (34.6, 67.3)
Summer	145	263	5.6 (4.2, 10.7)	5.8 (3.8, 10.9)	41.7 (32.0, 55.9)	76.3 (67.3, 87.3)	57.1 (44.4, 71.1)	53.0 (35.3, 84.2)	45.0 (34.8, 60.9)
Fall	147	261	6.0 (4.2, 13.5)	5.8 (4.1, 15.2)	43.9 (31.0, 59.2)	79.1 (69.2, 89.3)	57.1 (47.6, 72.7)	53.1 (37.5, 85.2)	46.3 (36.1, 60.0)
p-value[a]			0.72	0.53	0.20	0.50	0.13	0.54	0.25
Time of Day									
6– 8:59AM	145	300	5.3 (4.1, 8.4)	5.3 (3.8, 9.2)	38.2 (29.5, 50.0)	74.2 (64.4, 85.0)	55.4 (47.0, 66.7)	48.1 (33.3, 75.0)	44.8 (35.0, 5.4)
9 – 11:59AM	185	392	6.7 (4.5, 17.7)	6.7 (4.3, 19.0)	41.1 (31.1, 55.8)	80.0 (68.8, 90.4)	60.0 (46.2, 81.5)	64.5 (40.6, 92.5)	49.7 (36.0, 67.4)
12 – 2:59PM	131	152	5.6 (3.9, 10.7)	6.0 (3.7, 14.5)	45.2 (35.2, 60.7)	77.3 (68.2, 87.4)	60.2 (48.3, 81.0)	64.6 (36.0, 85.2)	46.2 (35.6, 68.3)
3 – 6PM	79	98	5.4 (4.1, 8.0)	4.6 (3.4, 8.5)	47.0 (35.3, 64.3)	77.4 (69.5, 87.4)	61.8 (50.0, 75.0)	56.1 (30.0, 87.7)	45.5 (36.1, 54.6)
p-value[a]			<0.0001	<0.0001	<0.0001	0.004	0.68	0.003	0.09

	# subjects	# samples	MEHP%	MECPP:MEHHP
Overall	269	943	5.2 (3.3, 8.2)	1.51 (1.19, 1.95)
Sex				
Female	157	688	5.3 (3.5, 8.3)	1.50 (1.19, 1.93)
Male	112	255	4.8 (2.9, 8.0)	1.53 (1.24, 1.97)
p-value[a]			0.07	0.36
Pregnant[b]				
No	157	565	5.1 (3.3, 7.9)	1.55 (1.22, 2.22)
Yes	75	123	6.3 (4.4, 9.8)	1.30 (1.05, 1.64)

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p-value[a]			<0.0001	<0.0001
Age				
<30	21	110	5.3 (3.3, 7.9)	1.48 (1.17, 1.93)
30-34	85	308	5.7 (3.2, 8.8)	1.53 (1.19, 2.04)
35-39	108	335	5.1 (3.6, 7.9)	1.52 (1.24, 1.91)
40-44	43	138	4.8 (3.1, 8.0)	1.46 (1.16, 1.89)
>=45	10	42	4.2 (3.0, 6.2)	1.25 (1.05, 1.83)
p-value[a]			0.18	0.83
BMI				
<24.9	134	511	5.6 (3.7, 8.9)	1.54 (1.21, 2.03)
25 – 29.9	83	252	4.4 (2.7, 7.1)	1.50 (1.18, 1.84)
>=30	48	155	5.1 (3.4, 7.1)	1.41 (1.13, 1.95)
p-value[a]			0.007	0.80
Race				
Non-white	34	100	6.5 (4.3, 9.1)	1.56 (1.19, 1.92)
White	233	833	5.0 (3.3, 8.1)	1.50 (1.20, 1.95)
p-value[a]			0.05	0.98
Smoking				
Non- Smoker	257	903	6.1 (4.2, 8.9)	1.51 (1.18, 1.96)
Smoker	12	40	5.6 (3.5, 8.3)	1.53 (1.32, 1.91)
p-value[a]			0.43	0.31
Season				
Winter	131	213	5.6 (3.5, 8.3)	1.46 (1.17, 1.89)
Spring	121	206	5.2 (3.3, 8.6)	1.53 (1.23, 2.04)
Summer	145	263	4.9 (3.4, 8.0)	1.50 (1.17, 1.96)
Fall	147	261	5.3 (3.2, 8.2)	1.53 (1.20, 1.98)
p-value[a]			0.47	0.48
Time of Day				
6– 8:59AM	145	300	4.8 (2.9, 7.0)	1.53 (1.23, 1.88)

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9 – 11:59AM	185	392	5.1 (3.4, 8.0)	1.60 (1.27, 2.08)
12 – 2:59PM	131	152	6.0 (3.2, 9.4)	1.46 (1.14, 1.95)
3 – 6PM	79	98	7.2 (4.4, 9.8)	1.18 (0.99, 1.51)
p-value[a]			<0.0001	<0.0001

[a]Test for fixed effects using log-transformed data in mixed models with random subject effects

[b]Female samples only.

[c]Measured in 519 samples from 176 people

[a]Test for fixed effects using log-transformed data in mixed models with random subject effects

[b]Female samples only.

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Table 5

Intraclass correlation coefficients (ICC) and 95% confidence intervals for (ln-transformed) urinary concentrations of free metabolites, total metabolites, and biotransformation indicators. Not corrected for specific gravity. N = 943 samples from 269 participants.

Metabolite	Form	ICC	95% Confidence Interval
MEHP	Total	0.13	0.08, 0.22
	MEHP%	0.39	0.32, 0.47
MEHHP	Free	0.08	0.04, 0.16
	Total	0.13	0.08, 0.21
	Free%	0.17	0.12, 0.25
	MECPP:MEHHP	0.26	0.19, 0.34
MEOHP	Free	0.10	0.05, 0.17
	Total	0.14	0.09, 0.21
	Free%	0.15	0.10, 0.23
MECPP	Free	0.15	0.10, 0.23
	Total	0.15	0.10, 0.23
	Free%	0.26	0.20, 0.33
MEP	Free	0.50	0.43, 0.57
	Total	0.49	0.42, 0.56
	Free%	0.22	0.15, 0.29
MCPP	Free	0.22	0.16, 0.30
	Total	0.24	0.18, 0.32
	Free%	0.04	0.01, 0.13
MCNP	Free	0.22	0.14, 0.34
	Total	0.23	0.14, 0.34
	Free%	0.15	0.08, 0.26
MCOP	Free	0.13	0.06, 0.25
	Total	0.14	0.07, 0.25
	Free%	0.11	0.05, 0.23